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THE INFLUENCE OF PROCESSING ON THE BIOACTIVE COMPOUNDS OF MULTIGRAIN FLOURS BASED ON WHEAT, RYE AND TRITICALE

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The multigrain flours obtained by milling with two different laboratory mills - blends of wheat, rye and triticale were characterized in terms of total phenolics content (TPC), antioxidant activity (DPPH-RSA) and thiamine content. Additionally, the influence of multigrain flours fermentation on the same nutritional properties of the obtained sourdough was studied. The TPC and DPPH-RSA of multigrain fractions obtained with the two mills followed the same trend. The amount of bioactive compounds in flours and brans increased with the level of rye and triticale within the multigrain blends, while in case of shorts the TPC and DPPH-RSA decreased. The multigrain flours obtained from Buhler laboratory mill had DPPH-RSA values about 1.75-2 times higher compared to the corresponding multigrain flours obtained with laboratory disc mill. The type of laboratory equipment used for multigrain milling influenced the distribution of thiamine rich particles in the multigrain fractions, the most important differences being registered in case of brans and shorts. The sourdough fermentation with a mixture of *Lactobacillus rhamnosus*, *Lactobacillus brevis* and *Lactobacillus plantarum* increased the levels of TPC, DPPH-RSA and thiamine contents for all multigrain flours. Moreover, lactic acid bacteria assisted sourdough fermentation led to a slight increase of the thiamine content compared to the control sourdoughs obtained through spontaneous fermentation. It can be concluded that the nutritional values of the bakery products could be modeled through multigrain milling with different types of grinding equipment and by the use of lactic acid bacteria for sourdough fermentation.

Keywords: *rye, triticale, processing, milling, sourdough, nutritional potential*

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Introduction

Alongside the macronutrients, cereals are able to provide the human diets significant quantities of micronutrients (Zieliński & Kozłowska, 2000). The content and bioavailability of the nutrients depends on the cereals nature but also on their processing. The multigrain milling could be a solution for increasing the micronutrients components of baked products. From this point of view, blending different amount of rye and triticale with the wheat, followed by milling results in multigrain blends that could have an important role in expanding the range of healthy baked products available on the market for consumers. The high nutritional value of rye, triticale and wheat is mainly due to the fibers, vitamins, minerals and different phytochemical compounds, such as phenolic acids that contribute to the antioxidant activity, alkylresorcinol and lignans (Shewry *et al*., 2013; Pihlava *et al*., 2015; Zhu, 2018). Several studies indicate important differences between rye, triticale and wheat in terms of concentration of total phenolic acids, their composition and the ratio between bound, conjugated and free fractions (Li *et al*., 2008; Jonnala *et al*., 2010; Pihlava *et al*., 2015). Moreover, the phenolic acids have a variable distribution in the anatomical parts of cereals (Zieliński & Kozłowska, 2000; Jonnala *et al*., 2010). Therefore, grains processing through milling can increase or decrease the total content of phenolic acids, playing an important role in the final quality of the baked products.

The type of mill and the flow diagram influence how the bran and germ are separated by the starchy endosperm. When high amounts of bran and germ are separated, the flour obtained from the starchy endosperm have lower amounts of micronutrient, because they are concentrated in bran and germ (Dewettinck *et al.,* 2008). Additionally, the type of force (e.g. shear, compression, or impact) used to break the grain for reaching the starchy endosperm and to further grind the starchy endosperm into flour can also influence the availability of nutrients (Sopade, 2017).

Lactic fermentation has been proved to be an efficient way to increase the nutritional value of cereal based products. Moreover, some lactic acid bacteria are able to diminish the negative impact of several anti-nutritional factors present in cereals. When considering the breadmaking process, the sourdough technology is a valuable tool that allows improving the availability of the bioactive compounds (Dewettinck *et al*., 2008). As reviewed by Lynch *et al*. (2018), sourdough fermentation might improve the mineral availability as a consequence of increasing the phytase activity, which might result in the release of different cations strongly bound to the phytic acid. The partial hydrolysis of different wheat peptides rich in proline and glutamine residues, which are responsible for inducing the autoimmune response in celiac patients, has been also mentioned (Lynch *et al*., 2018).

The objective of this study was to evaluate the influence of multigrain milling and of sourdough fermentation of multigrain flour, on the level of different biologically active compound from wheat, rye and triticale. In particular, the total phenolic contents, antioxidant activity and thiamine content of multigrain flours was monitored.

Materials and methods

Materials

Wheat (Boema variety), rye (Suceveana variety) and triticale (Oda FD variety) grown in South East Romanian Plain and harvested in 2016 were used in the experiments. A mixture of lactic acid bacteria consisting of *Lactobacillus rhamnosus*, *Lactobacillus brevis* and *Lactobacillus plantarum* (DI-PROX MTTX1, EDR Ingredients, Romania) was used as starter culture to prepare the sourdoughs.

Multigrain milling

Wheat (W), rye (R) and triticale (T) were blended in the ratio of 80:10:10 (80W+10R+10T), 70:15:15 (70W+15R+15T) and 60:20:20 (60W+20R+20T), followed the milling with two different mills: laboratory disc mill WZ-2 (Sadkiewicz Instruments, Poland) and Buhler laboratory mill MLU (Buhler, Uzwil, Switzerland). The milling processing was performed in agreement with the procedures described by Banu & Aprodu (2017) and Bolea *et al.* (2018), respectively. Regardless of the laboratory equipment used for multigrain milling, the following three milling streams were obtained: multigrain flour (F10, F15 and F20 resulting from multigrain blends with 10, 15 and 20% rye and triticale, respectively), bran (B10, B15 and B20 resulting from multigrain blends with 10, 15 and 20% rye and triticale, respectively) and short (S10, S15 and S20 resulting from multigrain blends with 10, 15 and 20% rye and triticale, respectively). The extraction rates of the multigrain flours resulted through milling the multigrain blends 80W+10R+10T, 70W+15R+15T and 60W+20R+20T varied with the mill as follows: 62% (F10_SK), 59.8% (15F_SK) and 56.5 (F20_SK), when the laboratory disc mill WZ-2 was used, and 77.9% (F10_B), 75.1% (F15_B) and 76.1% (F20_B), when Buhler laboratory mill MLU was used.

Sourdough preparation

The multigrain flours with different amounts of rye and triticale were further used to prepare sourdoughs. Flour suspensions were prepared in a large beaker by well mixing the multigrain flours with tap water in a ratio of 1:2. The flour suspensions were further inoculated with DI-PROX MTTX1 starter culture. The size of the inoculum used to initiate the fermentation was decided in agreement with producer recommendation. In order to obtain the sourdough (CS), the samples were then subjected to fermentation for 20 h at 30°C. For each type of investigated flour, control sourdoughs (C) were prepared without starter culture addition, through spontaneous fermentation for 20 h at 30ºC.

Table 1. Codification of the control and sourdough samples

Milling equipment	Multigrain flour	Samples	
		Control	Sourdough
Buhler laboratory mill MLU	F10_B	C10_B	CS10_B
	F15_B	C15_B	CS15_B
	F20_B	C20_B	CS20_B
Disc mill WZ-2	F10_SK	C10_SK	CS10_SK
	F15_SK	C15_SK	CS15_SK
	F20_SK	C20_SK	CS20_SK

Total phenolic content

The extraction of the phenolic compounds from the multigrain flours and sourdough samples was carried out using acidified methanol (HCl/methanol/water, 1:80:10, v/v/v) at room temperature for 2 h on a magnetic stirrer. After centrifugation for 15 min at 1000 x g using Hettich Universal 320 R centrifuge (Tuttlingen, Germany), the extract was used for quantifying the total phenolics content (TPC), using the Folin Ciocalteu method described by Singleton and Rossi (1965) and modified by Gao *et al*. (2002). The TPC was expressed as mg ferulic acid equivalent (FAE) per g d.w.

DPPH radical scavenging activity (DPPH RSA)

The extraction of the compounds with antioxidant activity was made by mixing the multigrain flours and sourdough samples with 80% aqueous methanol solution for 2 h at room temperature. The extract obtained through centrifugation for 15 min at 3000 rpm was used to assay the DPPH-radical scavenging activity (DPPH RSA) using the method described by Brand-Williams *et al*. (1995) and modified by Beta *et al*. (2005).

Thiamine content

The microbiological microtiter plate test was used to quantitate the vitamin B1 (thiamine) from multigrain flours and sourdough samples. The content of thiamine was determined using the VitaFast® Vitamin B1 (RBiopharm Rhone Ltd.) according to the procedure recommended by the manufacturer. After incubating for 44 h in the dark at 37°C, the optical density of the microtiter plate containing the mixture of diluted extract and assay medium was measured using the Stat Fax® 4700 plate reader (Awarness Technology, Inc.). The Optical density gives indication about the intensity of the thiamine related growth of *Lactobacillus fermentum* coated on the microtiter plate. The software RIDA® Soft Win (R-Biopharm AG, Germany) was used for quantification of the thiamine content.

Statistical analysis

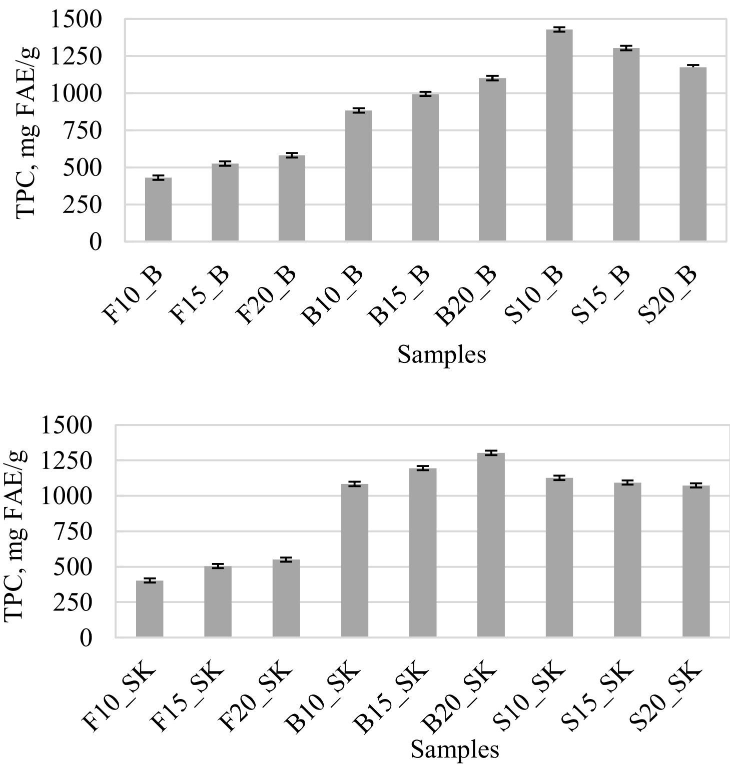
The experiments were carried out in triplicate, and the results are reported as average values together with standard deviation. Analysis of variance, performed with Microsoft Excel Soft, was used to identify significant differences.

Results and discussion

Influence of multigrain milling on the level of bioactive compounds in flours

The TPC of flour, bran and short streams resulted through multigrain milling varied with the type of experimental mill used in the study and with the percentages of rye and triticale within blends. As one can see in Figure 1, the TPC of the multigrain flours and shorts delivered by Buhler laboratory mill were higher than the corresponding mill streams obtained with the laboratory disc mill. On the other hand, higher TPC were found in the brans delivered by the laboratory disc mill. The increase of the addition level of rye and triticale in the multigrain blends resulted in the increase of TPC of flours and brans on the account of shorts.

According to Zhu (2018) the TPC of triticale is similar to that of wheat, but lower compared to rye. The most abundant phenolic acid in wheat, rye and triticale was reported to be ferulic acid (Jonnala *et al*., 2010; Pihlava *et al*., 2015; Zhu, 2018). Regarding the predominant location of the phenolic compound in the different layers of the kernels, several studies indicated that through milling wheat, rye and triticale, most of the TPC reaches the brand fractions, suggesting that they are manly concentrated in the pericarp and testa, rather than the endosperm (Zieliński & Kozłowska, 2000; Pihlava *et al*., 2015). In case of our experiment, the TPC in shorts collected from Buhler laboratory mill was higher compared to those delivered by the laboratory disc mill. These results indicate that the intensity of the grinding process varies with the milling equipment. When compared to the laboratory disc mill, the Buhler mill ensured a more intense grinding process, which resulted in higher amounts of particles originating mainly from pericarp and testa with lower size that end up into the shorts.



(a)

(b)

Figure 1. The total phenolic contents of milling fractions from Buhler laboratory mill (a) and from laboratory disc mill (b)

DPPH-RSA of the milling fractions (Figure 2) followed the same trend as TPC (Figure 1). The antioxidant activity of the mill streams is due to the phenolic acids and flavonoids that are found in higher amounts in bran fractions (Pihlava *et al*., 2015). The less intense grinding of the multigrain blends with the laboratory disc mill influenced the distribution of particles arising from pericarp and testa into the mill streams. In particular, the amounts of pericarp and testa particles reaching the brans are larger than those directed toward the shorts. The DPPH-RSA values of the same type of mill streams obtained by grinding the multigrain blends with different amounts of rye and triticale with the Buhler laboratory mill were rather similar. Anyway, important differences were observed between different types of mill streams (Figure 2a). Thus, the ratio between DPPH-RSA values of bran and short varied from 0.61 to 0.78 in case of fractions collected from the Buhler laboratory mill, and from 1.96 to 2.64 in case of fractions collected from the laboratory disc mill. The DPPH-RSA values of the multigrain flours obtained with Buhler laboratory mill were about 1.75-2 times higher compared to the multigrain flours resulted from the laboratory disc mill (Figure 2).

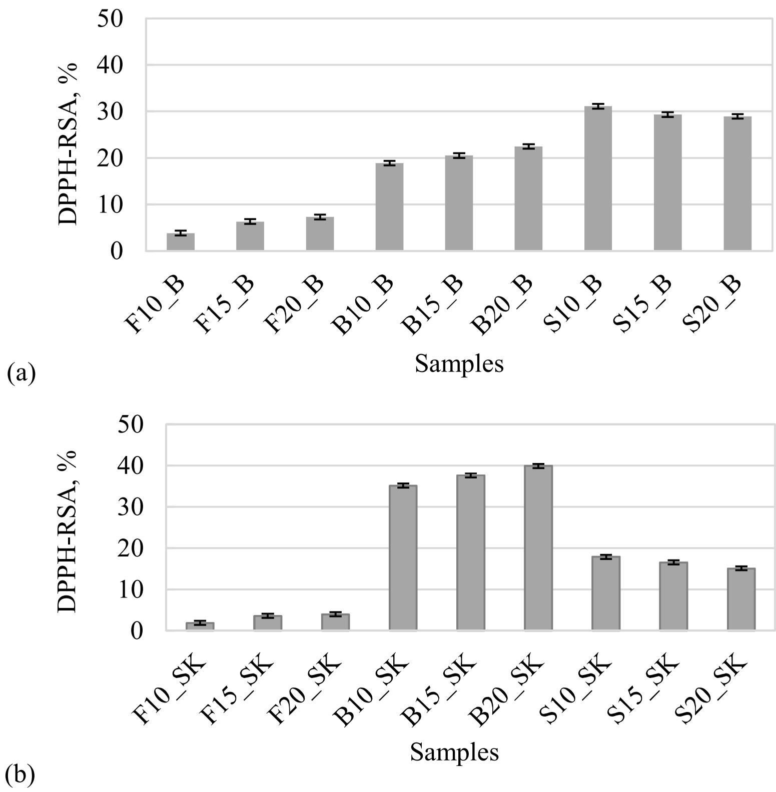


Figure 2. DPPH-RSA of milling fractions from Buhler laboratory mill (a) and from laboratory disc mill (b)

The thiamine contents of the fractions obtained through multigrain milling with the two laboratory equipment are presented in Figure 3. The wheat, rye and triticale had thiamine contents of 0.38 mg/100 g d.w, 0.32 mg/100 g d.w and 9.74 nmol/g, respectively (Capozzi *et al*., 2012; Zhu, 2018). According to Batifoulier *et al.* (2006), most of the thiamine content, representing about 80% of the total amount in cereals, is located in outer layer of the kernels.

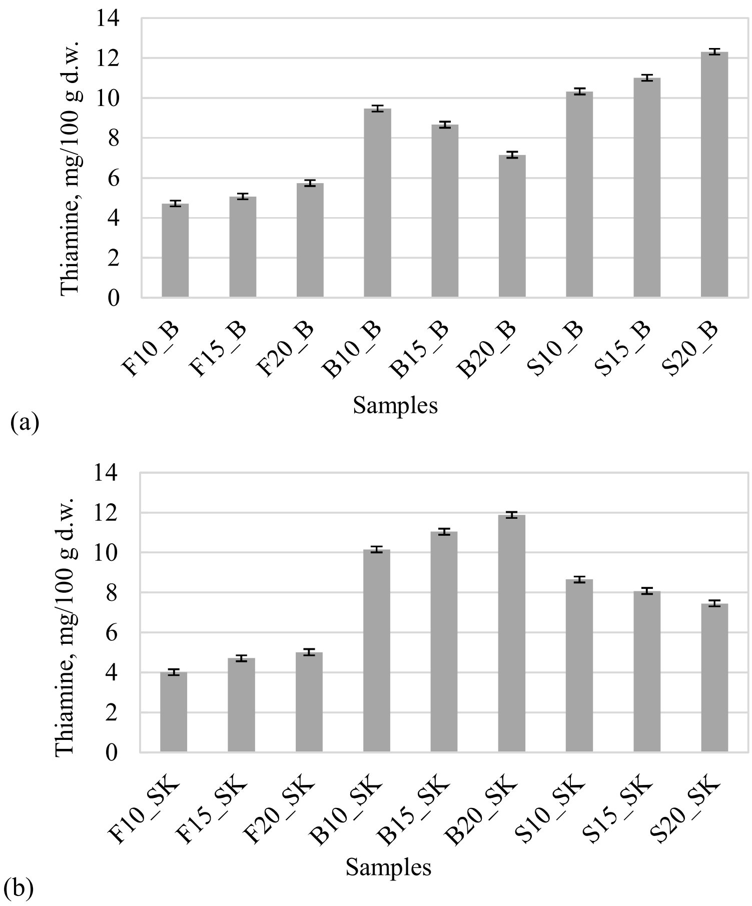
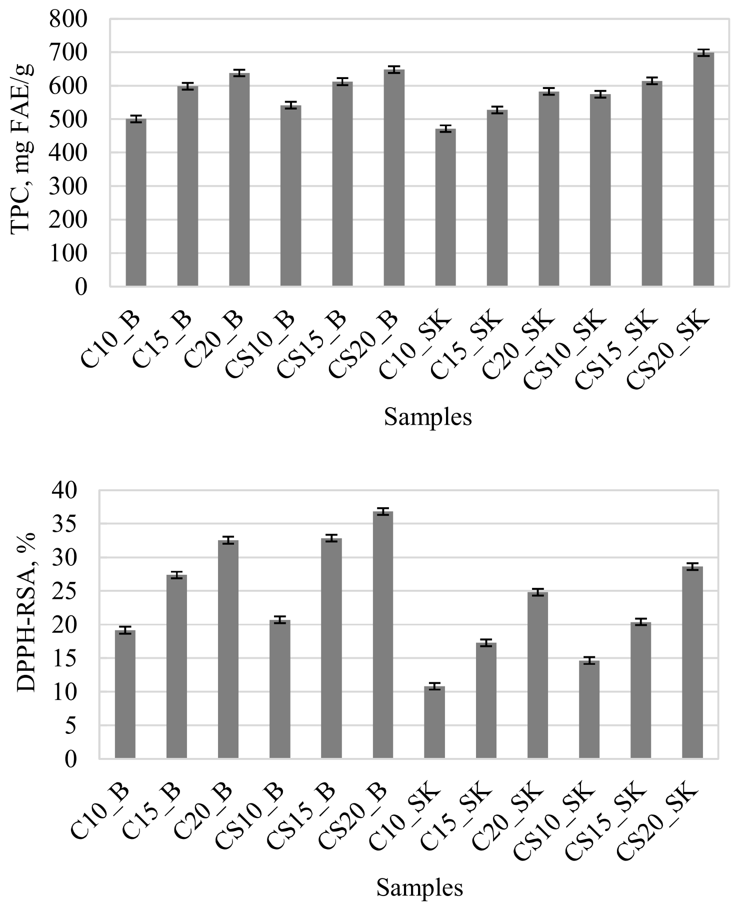


Figure 3. Thiamine contents of milling fractions from Buhler laboratory mill (a) and from laboratory disc mill (b)

Regardless of the type of mill used for grinding the multigrain blends, the thiamine content of the multigrain flours increased with the level of rye and triticale used to substitute the wheat within blends. Major differences among streams with different amounts of rye and triticale were noticed in case of the bran and shorts. When multigrain milling was performed with the Buhler laboratory mill, the thiamine contents increased from 10.32 to 12.31 mg/100 g d.w. in shorts and decreased from 9.47 to 7.15 mg/100 g d.w. in brans with the level of rye and triticale within blends (Figure 3a). A different trend was observed in the mill streams collected from the laboratory disc mill: the thiamine content in brans increased from 10.15 to 11.88 mg/100 d d.w. with the level of rye and triticale, on the account of shorts where the thiamine contents decreased from 8.65 to 7.45 mg/100 g d.w. Therefore, in case of grinding the multigrain blends with the Buhler laboratory mill brans were the fractions with the highest thiamine content, while in case of grinding with the laboratory disc mill the highest content of thiamine was found in shorts (Figure 3).

Influence of sourdough fermentation on the level of bioactive compounds

Sourdough fermentation was previously proved to be a suitable tool for increasing the level of bioactive compounds of flours (Banu *et al*., 2010). The TPC and DPPH-RSA of the sourdoughs fermented with and without starter culture addition are presented in Figure 4.



(a) (b)

Figure 4. The total phenolic contents (a) and antioxidant activity (b) of the sourdoughs prepared with (CS) and without starter culture (C)

The sourdough prepared out of multigrain flours resulted through grinding with the two laboratory mill and fermented with the aid of starter culture had higher TPC and DPPH-RSA values compared to the corresponding sourdough samples prepared through spontaneous fermentation. When comparing the sourdoughs prepared without starter culture, one can see that spontaneous fermentation of multigrain flours collected from Buhler laboratory mill resulted in higher TPC compared to the controls based on multigrain flours collected from the laboratory disc mill (Figure 4a). On the other hand, when starter culture was used for sourdough fermentation, the TPC were slightly higher in case of samples prepared with multigrain flours collected from laboratory disc mill compared to the multigrain flours collected from Buhler laboratory mill. Although the TPC of multigrain flours collected from the laboratory disk mill was lower compared to the Buhler mill (Figure 1) under the experimental conditions used in the study, it appears that fermentation carried out with *Lactobacillus rhamnosus*, *Lactobacillus brevis* and *Lactobacillus plantarum* might allow significant change of the bioactive compounds profile compared to the flour samples (Figure 4). Several studies indicated that some phenolic compounds from flours become more easily extractible through sourdough fermentation, therefore increasing the TPC values (Katina *et al*., 2007; Banu *et al*., 2010). Different enzymes originating from cereals, indigenous microbiota or added lactic acid bacteria are responsible for significant changes in the compositions of the sourdough samples, leading to improved extraction of the phenolic compounds as a consequence of releasing the bound phenolics (Katina *et al*., 2007; Đorđević *et al*., 2010).

Regardless of the starter culture addition for sourdough fermentation, the DPPH-RSA values were higher in case of samples prepared with multigrain flours from Buhler laboratory mill compared to those prepared with multigrain flours from laboratory disc mill. This trend is in good agreement with the one showing the bioactive compounds with antioxidant activity from the multigrain flours (Figure 2). In addition to the enhanced extraction of the chemical compounds with radical scavenging activity originating from the grains, the metabolic activity of lactic acid bacteria during sourdough fermentation might result in higher levels of exogenous bioactive compounds (Katina *et al*., 2007; Banu *et al*., 2010). In particular, Coda *et al*. (2012) showed that selected lactic acid bacteria are able to synthesize antioxidant peptides during sourdough fermentation.

Regarding thiamine contents in sourdoughs, our results indicated that samples prepared with multigrain flours from Buhler laboratory mill had higher vitamin contents compared to the flours resulted from laboratory disc mill (Figure 5).

Regardless of the equipment used for grinding and of starter culture addition, the thiamine content of sourdoughs increased with the levels of rye and triticale in the multigrain blends. According to Batifoulier *et al*. (2005), the fermentation with yeast leads to the decrease of thiamine content in bread. Anyway, they mentioned that longer fermentation time allowed improving the vitamin levels of the samples. Additionally, the authors reported in their study that in case of whole wheat bread the sourdough fermentation led to a level of thiamine close to the whole wheat flour. Our results highlighted that the sourdough fermentation with the starter culture leads to a slight increase of thiamine content compared to the multigrain flours. Moreover, compared to the control sourdoughs fermented without starter culture, the use of *Lactobacillus rhamnosus*, *Lactobacillus brevis* and *Lactobacillus plantarum* resulted in the increase of thiamine contents by 3-7% in case of sourdough samples prepared with multigrain flours from Buhler laboratory mill and by 13.7-15% for those prepared with multigrain flours resulted from laboratory disc mill (Figure 5).

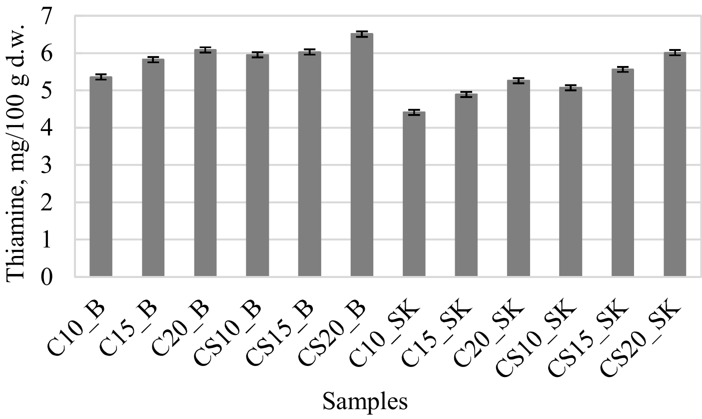


Figure 5. Thiamine contents of the sourdoughs prepared with (CS) and without starter culture (C)

Conclusions

The type of laboratory equipment used for multigrain milling influenced the distribution of TPC, DPPH-RSA and thiamine contents in milling fractions. When grinding was performed with the Buhler laboratory mill the highest TPC, DPPH-RSA and thiamine contents were obtained for brans, while in case of grinding with the laboratory disc mill the higher contents were obtained for shorts. The level of bioactive compounds of the multigrain flours increased with the percentage of rye and triticale within the blends. The sourdough fermentation of the multigrain flours increased the TPC, DPPH-RSA and thiamine contents. The highest increase of the bioactive compounds level was observed in case of sourdough prepared with multigrain flours collected from the laboratory disc mill.

Acknowledgments

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